

**Genetically Modified Live Attenuated  
Parasites as Leishmania vaccines :  
Evaluation of Safety and Efficacy**

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# Impact of Visceral Leishmaniasis



- 350 million are at risk worldwide
- 1-2 million new cases each year
- Estimated death toll of ~50,000/yr
- Drug Treatment unsatisfactory  
Painful, long (30 day)  
Expensive
- Emergence of Drug resistance

- Post Kala-azar Dermal L.
- Re-emergence w/ HIV
- T-T Transmission
- Mother to Child Transmission

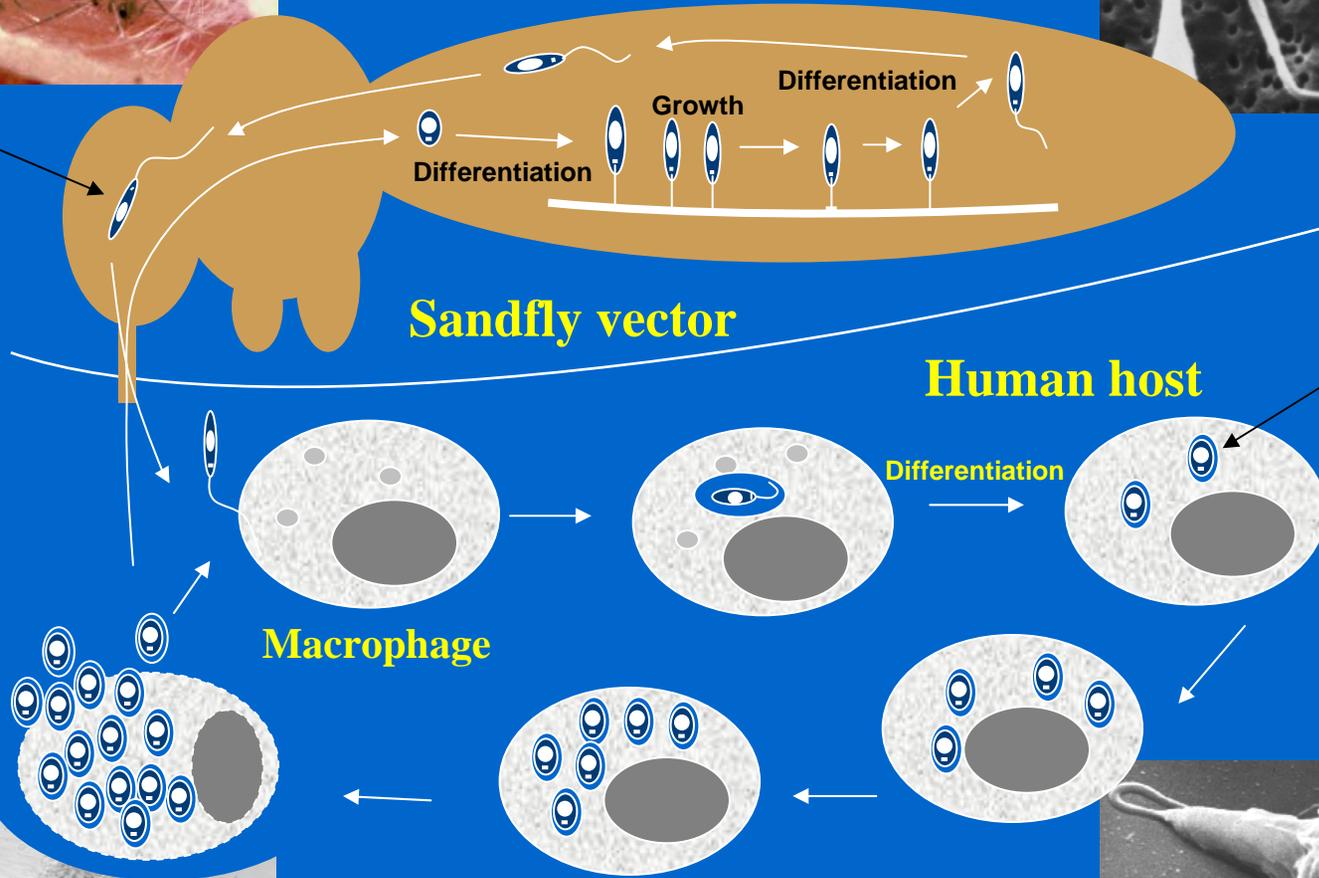
# Relevance to US Public Health

- Millions of American travelers and thousands of US troops are visitors or deployed in malaria, Chagas and *Leishmania* endemic areas (World is a global village)
- Increasing rates of immigration raises concern about the potential for transmission
- Known cases of Visceral *Leishmania* transmission through transfusion
- Significant number of potential donors are deferred based on potential exposure
- **No donor screening assays are available**
- **No vaccines are available**

# Life cycle of *Leishmania*



Promastigote



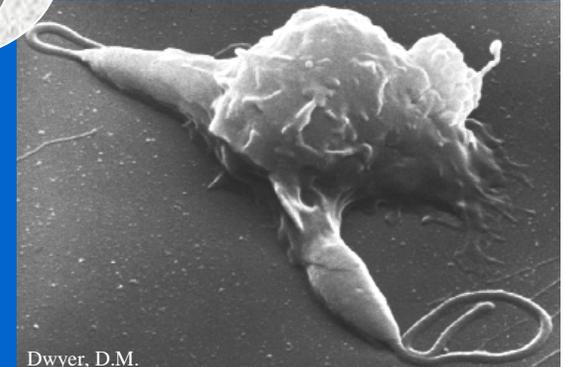
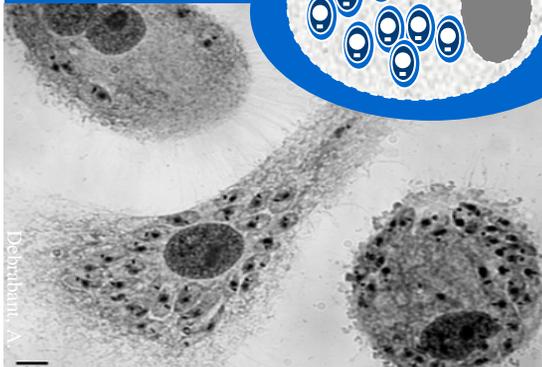
Sandfly vector

Human host

Amastigote

Macrophage

Adapted from Paulo Pimenta



Dwyer, D.M.

# Out come of previous studies on *Leishmania* vaccine development

## ■ Various strategies of vaccine development

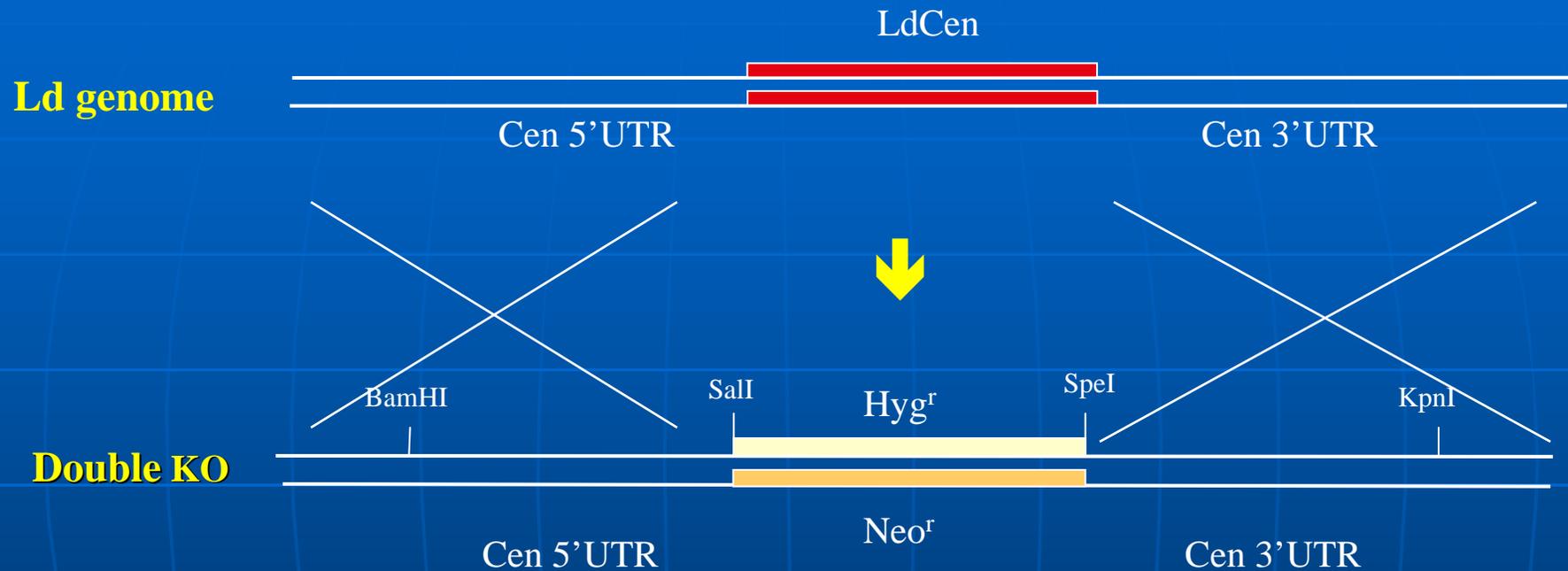
- Whole cell lysates/enriched fractions
- Attenuation through long term culture
- Chemical Mutagenesis
- Irradiated parasites
- Killed parasites
- Recombinant and Synthetic antigens + Adjuvant
- DNA vaccines and CpG ODNs
- All the above studies lead to the conclusion that parasite persistence may be required to maintain the immunological memory to prevent reinfection
  - Could be achieved by live-attenuated parasites immunization that could persist indefinitely w/o inducing disease

Selvapandiyan, et. al. 2006, Ind. J. Med. Res. 123: 455-466

# Specific Aims

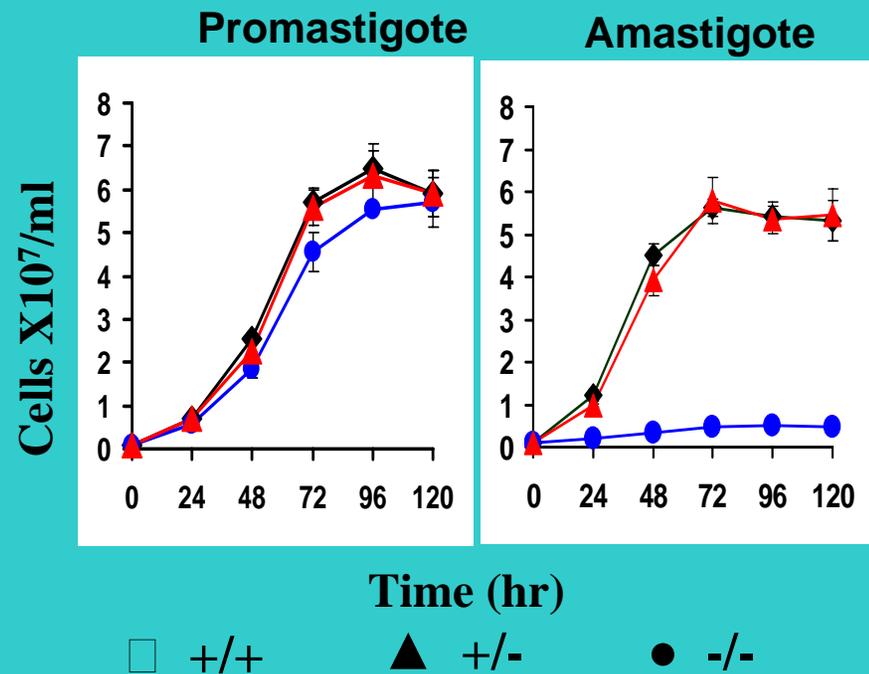
1. Develop **attenuated lines** of *Leishmania donovani* by defined genetic alteration
2. Assess the **immunogenicity** of genetically defined parasites
3. Identify **biomarkers** of genetic stability to monitor vaccine safety of live attenuated parasites

# Development of *L. donovani* parasite with deletion of growth controlling centrin gene



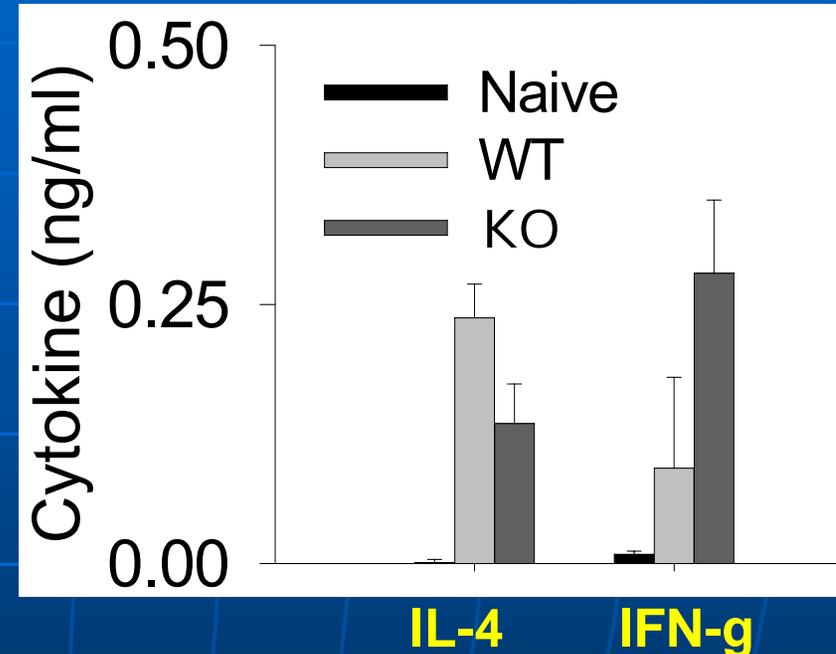
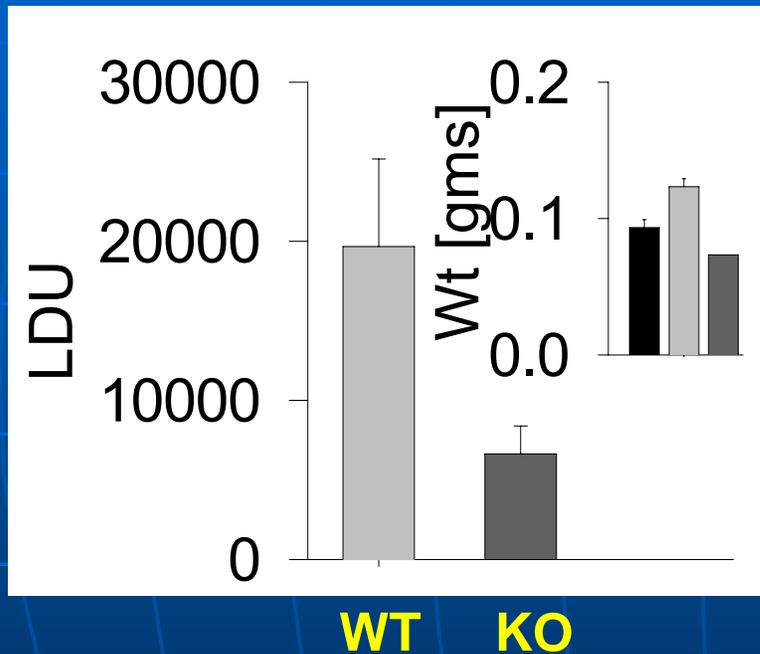
Selvapandiyan et. al. (2004) J. Biol. Chem. 279: 25702-10

# Summary



- The *Leishmania* centrin null mutants show differential growth arrest
- Axenic amastigote centrin null mutants are growth arrested in the G2/M phase and multi-nucleated.
- Duplication of Basal bodies is affected in the axenic amastigotes
- Centrin KO amastigotes do not survive in the macrophages
- ***Leishmania* centrin null mutants are potential attenuated vaccine candidates**

# Immunogenicity and pathogenicity of centrin null mutants of *L. donovani* in BALB/c mice

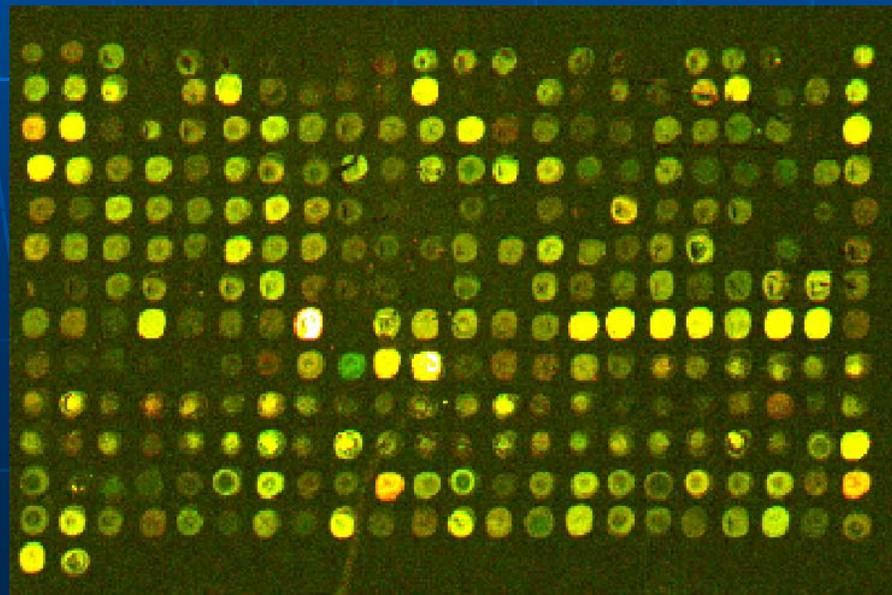


1. Reduced Survival of KO parasites
2. Reduced Pathogenicity of KO parasites
3. Infection with KO parasites resulted in a Th1 (IFN-g) predominated antileishmanial T cell response

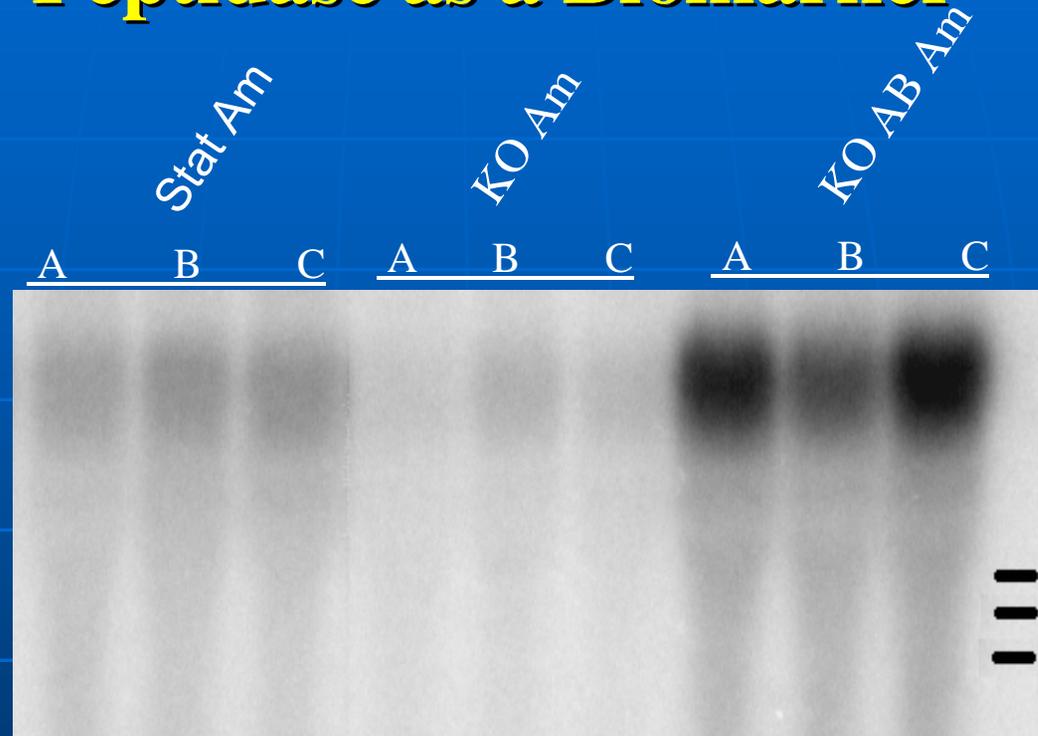
**Saha et al., (unpublished data)**

# Identification of Biomarkers to monitor vaccine safety of live attenuated centrin null mutant *Leishmania*

- A microarray of 4224 clones printed in triplicate.
- Axenic Amastigote samples of *Ldcen*<sup>+/+</sup> RNA and of *Ldcen*<sup>-/-</sup> RNA were used for hybridization to the microarray
- Differentially expressed genes were identified



# Expression Pattern of Calpain-like Cysteine Peptidase as a Biomarker



calpain-like cysteine peptidase

1. Expression is significantly reduced in KO vs wild type parasite
2. Expression is directly related to centrin modification
3. Expression of Calpain-like cysteine peptidase in Centrin KO parasites in vaccinated animals can be used to monitor for its genetic stability

# Conclusions and Future Directions

- Centrin deleted parasites can be exploited in the development of an attenuated *Leishmania* parasite
  - Disruption of centrin gene results in growth arrest in vitro and in vivo
  - Reduced survival and pathogenicity in animal model
  - Induction of antileishmanial immune response
- Assess the **immunogenicity** of centrin deleted attenuated parasites in small and large animal models
- Characterize genes identified through microarray analysis as biomarkers to monitor the **genetic stability** of the centrin-deleted live attenuated vaccine.
- Develop additional genetically defined **live attenuated** *L. donovani* parasites to be tested as vaccine candidates

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